IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

First Named Inventor:	LINDSTROM, KYLE J.		
Application No.:	10/669,015	Group Art Unit:	1625
Filed:	September 23, 2003	Examiner:	Huang, Evelyn Mei
Title:	SULFONAMIDE AND SULFAMIDE SUBSTITUTED IMIDAZOQUINOLINES		

AFFIDAVIT UNDER 37 C.F.R. § 1.132

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December 17, 2004 — Virginia Bergstrom
Date — Signed by: Virginia Bergstrom

Dear Sir:

I, Dr. Bryon A. Merrill, being duly sworn, do hereby depose and state that:

1. I hold a Bachelor of Science Degree in Chemistry, granted to me from the University of Wisconsin – Green Bay in 1983.

2. I hold a Doctorate in Organic Chemistry from Michigan State University, granted to me in 1990.

3. I was employed as a National Institutes of Health Postdoctoral Fellow at the University of California – Irvine from 1990 – 1992.

4. I have been employed at 3M Company in St. Paul, Minnesota since 1992 for the purpose of conducting research.

5. Since 1992, I have been employed at 3M in the Pharmaceuticals Business Unit in a capacity for research and development of new pharmaceutical compounds.

6. I have read and am familiar with the patent application identified as U.S. Application No. 10/669,051.

7. Using the "CYTOKINE INDUCTION IN HUMAN CELLS" assay described on pages 125-126 of the specification (of Application No. 10/669,051), the compound of the invention, N-[4-(4-amino-2-ethyl-1*H*-imidazo[4,5-*c*]quinolin-1-yl)butyl]methanesulfonamide (in

U.S. Application No. 10/669,051), and Crooks' compound, N-[4-(4-amino-2-butyl-1*H*-imidazo[4,5-*c*]quinolin-1-yl)butyl]methanesulfonamide (in U.S. Patent No. 6,331,539), were tested at the same time, at the same concentration, with the same reagents, including the same human PBMCs to determine the amounts of interferon α (IFN-α) and tumor necrosis factor α (TNF-α) induced by each compound. The tests were carried out at a number of concentrations of the test compounds. These experiments were run multiple times.

8. Unexpectedly, the compound of the invention was found to induce much less TNF-α than that induced by Crooks' compound at all test concentrations. Amounts of TNF-α induced by each compound are shown in the following Tables 1-3.

9. Table 1

	Amount of TNF-α produced (pg/ml) at a 3.3 μM concentration of test compound:	
Experiment No.	N-[4-(4-amino-2-ethyl-1*H*-imidazo[4,5-*c*]quinolin-1-yl)butyl]methanesulfonamide	N-[4-(4-amino-2-butyl-1*H*-imidazo[4,5-*c*]quinolin-1-yl)butyl]methanesulfonamide
3571	213	1808
3597	148	1827
3603	163	6812
3050	469	1674
3056	496	5080
3106	360	1030
4166	53	575
3065	251*	1057**

10. To summarize the results from Table 1, at 3.3 μM the average amount of TNF-α produced by N-[4-(4-amino-2-butyl-1*H*-imidazo[4,5-*c*]quinolin-1-yl)butyl]methanesulfonamide was 2483 pg/ml with a range of (575 to 6812 pg/ml), while at 3.3 μM the average amount of TNF-α produced by N-[4-(4-amino-2-ethyl-1*H*-imidazo[4,5-*c*]quinolin-1-yl)butyl]methanesulfonamide was only 269 pg/ml with a range of (53-496 pg/ml)

11. Table 2

	Amount of TNF-α produced (pg/ml) at a 1.1 μM concentration of test compound:	
Experiment No.	N-[4-(4-amino-2-ethyl-1*H*-imidazo[4,5-*c*]quinolin-1-yl)butyl]methanesulfonamide	N-[4-(4-amino-2-butyl-1*H*-imidazo[4,5-*c*]quinolin-1-yl)butyl]methanesulfonamide
3571	59	1641
3597	67	881
3603	143	3311
3050	106	2101
3056	84	6111
3106	91	836
4166	12	352
3065	61*	752**

12. To summarize the results from Table 2, at 1.1 μM the average amount of TNF-α produced by N-[4-(4-amino-2-butyl-1*H*-imidazo[4,5-*c*]quinolin-1-yl)butyl]methanesulfonamide was 1998 pg/ml with a range of (352 to 6111 pg/ml), while at 1.1 μM the average amount of TNF-α produced by N-[4-(4-amino-2-ethyl-1*H*-imidazo[4,5-*c*]quinolin-1-yl)butyl]methanesulfonamide was only 78 pg/ml with a range of (12-143 pg/ml)

13. Table 3

	Amount of TNF-α produced (pg/ml) at a 0.37 μM concentration of test compound:	
Experiment No.	N-[4-(4-amino-2-ethyl-1*H*-imidazo[4,5-*c*]quinolin-1-yl)butyl]methanesulfonamide	N-[4-(4-amino-2-butyl-1*H*-imidazo[4,5-*c*]quinolin-1-yl)butyl]methanesulfonamide
3571	9	442
3597	28	363
3603	112	1029
3050	43	1068
3056	27	3343
3106	79	616

4166	4	110
3065	27*	339**

14. To summarize the results from Table 3, at 0.37 μM the average amount of TNF-α produced by N-[4-(4-amino-2-butyl-1*H*-imidazo[4,5-*c*]quinolin-1-yl)butyl]methanesulfonamide was 914 pg/ml with a range of (110 to 3343 pg/ml), while at 0.37 μM the average amount of TNF-α produced by N-[4-(4-amino-2-ethyl-1*H*-imidazo[4,5-*c*]quinolin-1-yl)butyl]methanesulfonamide was only 41 pg/ml with a range of (4-112 pg/ml)

15. This finding is of considerable significance. TNF-α is a pro-inflammatory cytokine, and although high levels of TNF-α are desirable for the treatment of some diseases, in many circumstances it can be undesirable such that lower levels are advantageous.

16. Furthermore, the compound of the invention was unexpectedly found to induce the production of significantly more IFN-α than that induced by Crooks' compound. This was found to be true over a range of concentrations, including 3.3, 1.1, and 0.37 μM. The amounts of IFN-α induced by each compound are shown in the following Tables 4-6.

17. Table 4

	Amount of IFN-α produced (pg/ml) at a 3.3 μM concentration of test compound:	
Experiment No.	N-[4-(4-amino-2-ethyl-1*H*-imidazo[4,5-*c*]quinolin-1-yl)butyl]methanesulfonamide	N-[4-(4-amino-2-butyl-1*H*-imidazo[4,5-*c*]quinolin-1-yl)butyl]methanesulfonamide
3571	3482	366
3597	6660	166
3603	1379	545
3050	595	627
3056	144	137
3106	2636	1572
4166	1052	1017
3065	243*	895**

18. The results in Table 4 show that at the 3.3 μM concentration N-[4-(4-amino-2-ethyl-1*H*-imidazo[4,5-*c*]quinolin-1-yl)butyl]methanesulfonamide induced the production of

significantly higher levels of IFN-α than that induced by N-[4-(4-amino-2-butyl-1*H*-imidazo[4,5-*c*]quinolin-1-yl)butyl]methanesulfonamide in 4 of 8 experiments. In 1 of 8 experiments, N-[4-(4-amino-2-butyl-1*H*-imidazo[4,5-*c*]quinolin-1-yl)butyl]methanesulfonamide induced the production of significantly higher levels of IFN-α than that induced by N-[4-(4-amino-2-ethyl-1*H*-imidazo[4,5-*c*]quinolin-1-yl)butyl]methanesulfonamide. For these experiments a significant difference is defined as a greater than 50% increase in the amount of IFN-α produced.

19. Table 5

	Amount of IFN-α produced (pg/ml) at a 1.1 μM concentration of test compound:	
Experiment No.	N-[4-(4-amino-2-ethyl-1*H*-imidazo[4,5-*c*]quinolin-1-yl)butyl]methanesulfonamide	N-[4-(4-amino-2-butyl-1*H*-imidazo[4,5-*c*]quinolin-1-yl)butyl]methanesulfonamide
3571	11210	333
3597	13545	182
3603	7968	604
3050	935	559
3056	418	133
3106	3315	1850
4166	2313	862
3065	634*	361**

20. The results in Table 5 show that at the 1.1 μM concentration N-[4-(4-amino-2-ethyl-1*H*-imidazo[4,5-*c*]quinolin-1-yl)butyl]methanesulfonamide induced the production of significantly higher levels of IFN-α than that induced by N-[4-(4-amino-2-butyl-1*H*-imidazo[4,5-*c*]quinolin-1-yl)butyl]methanesulfonamide in 8 of 8 experiments. For these experiments a significant difference is defined as a greater than 50% increase in the amount of IFN-α produced.

21. Table 6

	Amount of IFN-α produced (pg/ml) at a 0.37 μM concentration of test compound:	
Experiment No.	N-[4-(4-amino-2-ethyl-1*H*-imidazo[4,5-*c*]quinolin-1-yl)butyl]methanesulfonamide	N-[4-(4-amino-2-butyl-1*H*-imidazo[4,5-*c*]quinolin-1-yl)butyl]methanesulfonamide
3571	8832	1167
3597	15759	1482

3603	6160	414
3050	1537	471
3056	607	94
3106	2463	2200
4166	2486	739
3065	743*	228**

22. The results in Table 6 show that at the 0.37 μM concentration N-[4-(4-amino-2-ethyl-1*H*-imidazo[4,5-*c*]quinolin-1-yl)butyl]methanesulfonamide induced the production of significantly higher levels of IFN-α than that induced by N-[4-(4-amino-2-butyl-1*H*-imidazo[4,5-*c*]quinolin-1-yl)butyl]methanesulfonamide in 7 of 8 experiments. For these experiments a significant difference is defined as a greater than 50% increase in the amount of IFN-α produced.

23. For Tables 1-6 the value listed with one asterisk (*) represents the average of three independent measurements conducted in the same experiment. For Tables 1-6 the value listed with two asterisks (**) represents the average of two independent measurements conducted in the same experiment.

24. In summary, the difference between the cytokine induction profiles of N-[4-(4-amino-2-ethyl-1*H*-imidazo[4,5-*c*]quinolin-1-yl)butyl]methanesulfonamide and N-[4-(4-amino-2-butyl-1*H*-imidazo[4,5-*c*]quinolin-1-yl)butyl]methanesulfonamide described above is a useful and unexpected difference between the two compounds. In the concentration range of 3.3 μM - 0.37 μM, the compound of the invention induced higher levels of IFN-α production, but lower levels of TNF-α production than Crooks' compound. These important differences could not have been predicted based on the structures of these compounds.

25. The compound of the invention has been found to possess even further unexpected properties. The compound of the invention and the Crooks' compound were directly compared in an in vitro assay designed to determine the potential of a compound to produce changes in cardiac rhythm. In this test the concentration of compound required to inhibit 50% of HERG potassium channels (IC50) was determined. The HERG potassium channel inhibition assay is used as a model to predict the potential of a compound to prolong the repolarization of cardiac cells (i.e. QT interval). Drug-induced QT interval prolongation is associated with sudden cardiac death.

26. The assay was run as follows. Human ERG (HERG) -transfected Chinese Hamster Ovary (CHO) cells were plated into a 96-well microplate and incubated overnight. Cells were loaded with RbCl (5.4 mM) and incubated at 37°C for 1 hr. Excess RbCl was removed by washing three times with KCl buffer (5.4 mM). The test compound and test inhibitor (E-4031) were added separately at various concentration levels and the cells further incubated at 37°C for 10 min. The HERG channels were activated by adding elevated extracellular K^+ (60 mM). The supernatant (SN) was removed and collected, while the cell pellet was resuspended with lysis buffer (1% (v/v) Triton X-100). Rb^+ concentrations in cell supernatant and cell lysate fractions were quantitated by atomic absorption spectroscopy (Ion Channel Reader (ICR) 8000 (Aurora Biomed Inc., Vancouver, B.C.)). Rb^+ efflux rate was calculated using: $[Rb^+]SN/[Rb^+]Total$. This is the IC50 value. Because this is an assay to measure the interaction of test compound with potassium channels, the higher the IC50 value, the less chance there is for an undesired cardiac rhythm change. The results from the assay were as follows:

compound of the invention: HERG IC50 = 29 μM

Crooks' compound: HERG IC50 = 7.7 μM

Thus, the compound of the invention is distinguished from the Crooks' compound based on interaction with potassium channels.

27. The compound of the invention and the Crooks' compound were also directly compared with regard to metabolic stability (i.e. resistance to compound modification in the liver). In an in vitro assay, a set amount of test compound was exposed to a set amount of human liver microsomes, and the time required for half of the test compound to be metabolized (t1/2) was determined.

28. The assay was carried out as follows. Human liver microsomes (pooled from multiple human livers) were incubated at 37 °C in the presence of 1 μM test compound and an NADPH-regenerating system. Samples are quenched at 0, 10, and 60 minutes. Amount of test compound (peak area) remaining relative to 0 minute was determined by LC/MS/MS. Peak areas were natural log transformed and plotted vs. time. The rate of compound disappearance was determined by linear regression and the half-life (t1/2) calculated from rate. The results were as follows:

compound of the invention: t1/2 = > 240 minutes

Crooks' compound: t1/2 = 100 minutes

29. A compound with a longer t1/2 is more slowly metabolized and is a better candidate for oral dosing. That is, a longer t1/2 indicates that the compound is less likely to be removed prior to entering systemic circulation by the "first-pass effect" and has greater metabolic stability. Thus, the compound of the invention possesses much greater metabolic stability than Crooks' compound.

30. These findings clearly show that the compound of the invention possesses unexpectedly improved properties over those of Crooks' compound.

Further Affiant sayeth not.

State of Minnesota)
) ss.
County of Ramsey)

Bryon A. Merrill

Sworn to and subscribed before me by
the above-named applicant this
17 day of December, 2004.

Virginia Bergstrom
Notary Public

VIRGINIA L. BERGSTROM
NOTARY PUBLIC - MINNESOTA
My Commission Expires Jan. 31, 2005